Supporting Information

Injectable, biomolecule-responsive polypeptide hydrogels for cell encapsulation and facile cell recovery through triggered degradation

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S1. Materials and Methods

S1.1 Chemicals and Reagents

Cystamine dihydrochloride (Shanghai Darui Jinxi Chemical Ltd, China), di-tert-butyl dicarbonate (Adamas Reagent Co., Ltd), phloretic acid (Aladdin), tyramine (Energy Chemical), Glutathione (GSH, Aladdin), *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, GL Biochem), *N*-hydroxysuccinimide (NHS, GL Biochem) and horseradish peroxidase (HRP, 250 units/mg, Sigma-Aldrich) were used without further purification. *N*, *N*-dimethylformamide (DMF) was stored over CaH_2 and distilled under vacuum before use. All the other reagents and solvents were used as received unless otherwise stated. γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG NCA) was synthesized according to the previously reported procedure.[1]

S1.2 Characterization

^{1}H NMR spectra were recorded on a Bruker AV 400 NMR spectrometer. Molecular weights (M_W) and polydispersity indexes (PDI) were determined by gel permeation chromatography (GPC) equipped with a waters linear Ultrahydrogel column and a Waters 1515 isocratic HPLC pump with a Waters 2414 refractive index detector. The eluent was 0.1 M phosphate buffer (PB) containing 0.1 M NaN_3 at a flow rate of 1.0 mL min^{-1} at room temperature. Monodispersed PEG purchased from Waters Co. with the molecular weight ranging from 3000 to 1.0×10^5 was used as standards to generate the calibration curve. The number of phenol groups conjugated to the side chain of poly(L-glutamate) (PLG) was measured by using ultraviolet-visible (UV-Vis) spectrometer (UV-2401PC, Shimadzu, Kyoto, Japan). The PLG-*g*-CPA copolymer

was dissolved in distilled water with the concentration of 1 mg mL^{-1} and the absorbance at 275 nm was measured. The absorbance of tyramine hydrochloride dissolved in distilled water with different concentrations was measured to obtain the calibration curve and the content of conjugated phenol groups was calculated based on the calibration curve. The secondary structure of the copolymer was measured using a JASCO J-810 spectrometer in deionized water (0.1 mg mL^{-1}) at 25 °C.

S1.3 Gelation Time and Rheological Property of the PLG-*g*-CPA Hydrogels

The enzymatically-crosslinked hydrogels were prepared in presence of H_2O_2 and HRP at room temperature. The sol-gel phase transition was recorded by the vial inverting method. To evaluate the dependence of gelation time on the concentration of HRP, the PLG-*g*-CPA polymer solutions in 0.01 M PBS (pH 7.4) with different concentrations (1.5 %, 3%, 4.5%, 6% (w/v), 200 μL) were mixted with H_2O_2 (0.3 in molar ratio with CPA, 50 μL) and different concentrations of HRP (50 μL) in the test vial with the inner diameter of 11 mm. The mixtures with final polymer concentrations of 1%, 2%, 3% and 4% (w/v) were considered to be a gel state in the case of no flow observed within 30 s. Additionally, the PLG-*g*-CPA polymer solutions with the addition of HRP or H_2O_2 only were also tested by the vial inverting method to see if the polymer solution could transfer to hydrogel.

Rheological properties of the PLG-*g*-CPA hydrogels were recorded on a MCR 301 rheometer (Anton Paar) by using the parallel plates of 25 mm in diameter with a gap of 0.5 mm in oscillatory mode. Before each measurement, the polymer solution was quickly mixed with HRP and H_2O_2 solution by vortex, and the mixture was

immediately placed between the plates of the rheometer. A thin layer of silicone oil was put around the edge of the sample to prevent water evaporation. The storage modulus (G') and loss modulus (G'') were collected as a function of time at 37 °C with a strain of γ 1% and a frequency of 1 Hz.

S1.4 Swelling Behavior of the Hydrogels

PLG-*g*-CPA and PLG-*g*-TA hydrogels were prepared in vials by mixing the polymer solution (200 μL) with HRP (50 μL, final concentration: 1 unit/mL) and H_2O_2 solution. The mixture was incubated at room temperature for 30 min and the formed hydrogel was then freeze-dried. The dry hydrogels were accurately weighed (W_0) and then incubated in 10 mL of PBS solution containing different concentrations of GSH at 37 ° C. At predetermined time intervals, the media were completely removed and the remaining hydrogels were weighed (W_t). The swelling ratio (SR) was calculated from the equation: SR = (W_t - W_0)/ W_0. The experiment was performed in triplicate.

S1.5 *In Vitro* Cytocompatibility

In vitro cytocompatibility of PLG-*g*-CPA copolymer was assessed by the MTT assay against mouse fibroblast L929 cells. L929 cells were seeded in 96-well plates at the density of 10000 cells per well and cultured with 200 μL DMEM containing 10% fetal bovine serum, 50 U/mL streptomycin and 50 U/mL penicillin. Cells were incubated at 37 ^{o}C in 5% CO_2 atmosphere for 24 h, and then the cells were cultured for another 24 h with fresh media containing PLG-*g*-CPA at a series of concentrations (0.0625 - 1.0 g L^{-1}). After that, the media was removed and fresh media were added followed by adding of MTT (20 μL per well). After 4 h of incubation, the precipitated formazan

was dissolved in DMSO (200 μL per well) and the absorbance at 490 nm was measured using a Bio-Rad 680 microplate reader. Cells cultured without the copolymer was set as control and cell viability (%) was calculated by comparing the absorbance values with that of control groups. Additionally, the cytocompatibility of the extracts of PLG-*g*-CPA and PLG-*g*-TA hydrogels was also evaluated in a similar procedure. The hydrogels (600 μL) were leached by using DMEM (3 mL) for 1, 3 and 5 days, respectively. Cells were cultured with the collected eluants for 24 h, and cell viability were tested by MTT assay.

L929 cells were encapsulated into the PLG-*g*-CPA hydrogels (4% (w/v)) and cultured in three dimensional (3D) to elucidate the cytocompatibility of the hydrogels. First, PLG-*g*-CPA solution (6 % (w/v), 200 μL) was mixed with harvested L929 cells (1×10^5 cells), and then HRP and H_2O_2 solutions (50 μL for each) were added. The mixture was subsequently placed into the well of a 24-well plate after gently shaken. DMEM was added (1 mL per well) after the formation of the hydrogel. After cultured for 6 h and 24 h, the viability of the encapsulated cells was measured by the cell counting kit-8 (CCK-8) method. CCK-8 solution (0.5 mL per well, 10% (v/v) in medium) was added at predetermined period and incubated for 4 h. Then the absorbance at 450 nm was measured with an ELISA reader (Model 550; Bio-Rad, Hercules, USA), and the absorbance value at 630 nm was used as baseline correction (n = 3). In addition, cell viability was also evaluated by using a Live/Dead cell staining kit. Briefly, 0.5 mL of PBS containing 2.0 μM calcein AM and 4.0 μM propidium iodine (PI) was added into each well and incubated for 30 min. Viable cells

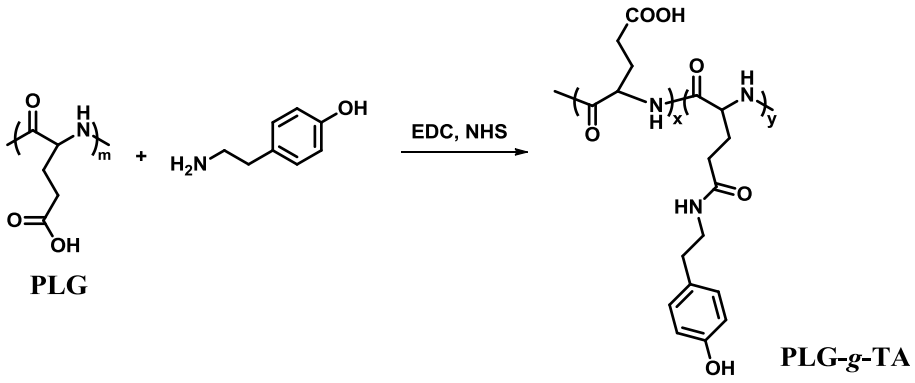
were stained green by calcein AM while dead cells were stained red by PI.

In addition, to see if the cells were cultured in 3D environment after encapsulated into the PLG-*g*-CPA hydrogel, cells were stained with DAPI (0.1% v/v, 5 min) after cultured for 2 h in the hydrogel. The stacked 3D image and the longitudinal section image of the cell/hydrogel constructs were observed by using 10× magnification on the confocal laser scanning microscope (CLSM, Carl Zeiss, LSM 700). The thickness of the hydrogel used for stacked 3D image was about 380 μm, and it was about 5 mm for the longitudinal section image. The cell density was 1×10^6/mL in the hydrogel.

S1.6 *In Vivo* Degradation and Biocompatibility of the Hydrogels

The animal experiments were carried out according to the guide for the care and use of laboratory animals, provided by Jilin University, Changchun, China, and the procedure was approved by the local Animal Ethics Committee. The PBS solutions of PLG-*g*-CPA (4% (w/v), 0.5 mL for each sample) together with HRP (1 unit/mL) and H_2O_2 (5 mM) were injected subcutaneously into the back of Sprague-Dawley (SD) rats (~ 200 g). The PLG-*g*-TA hydrogels were injected with the similar method. The rats were euthanized at predetermined time intervals (15 min as well as 3, 6 and 12 days), and status of the hydrogels were observed. In addition, the tissue next to the hydrogels were surgically removed and examined by hematoxylin and eosin (H&E) staining to evaluate the inflammatory responses of rats to the hydrogels.

Scheme S1. Synthetic route of PLG-*g*-TA copolymer.



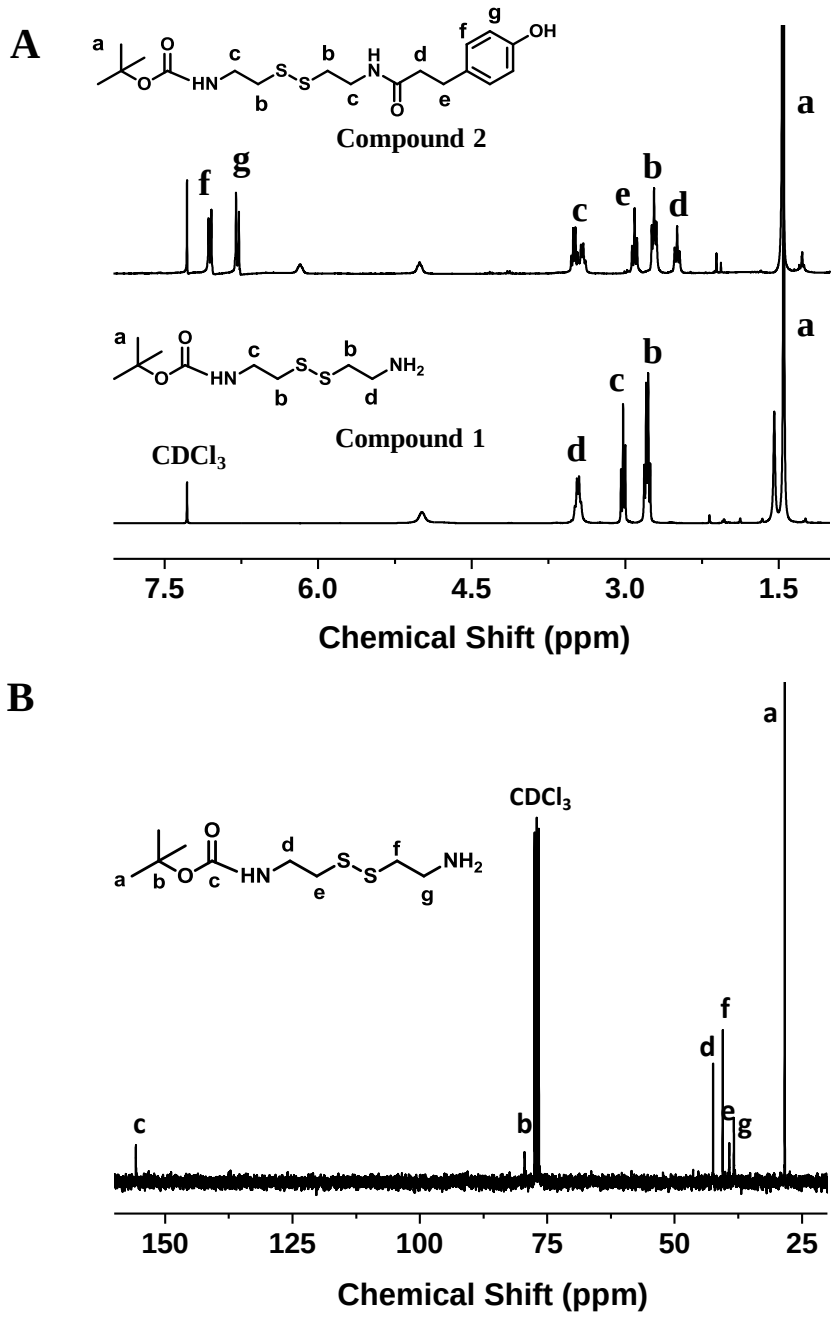


Figure S1. (A) ^{1}H NMR spectra of the two intermediate products (**compound 1** and **compound 2** as showed in **Scheme 1**) in $CDCl_3$ during the synthesis of disulfide bond modified phloretic acid (CPA). (B) ^{13}C NMR spectrum of **compound 1**.

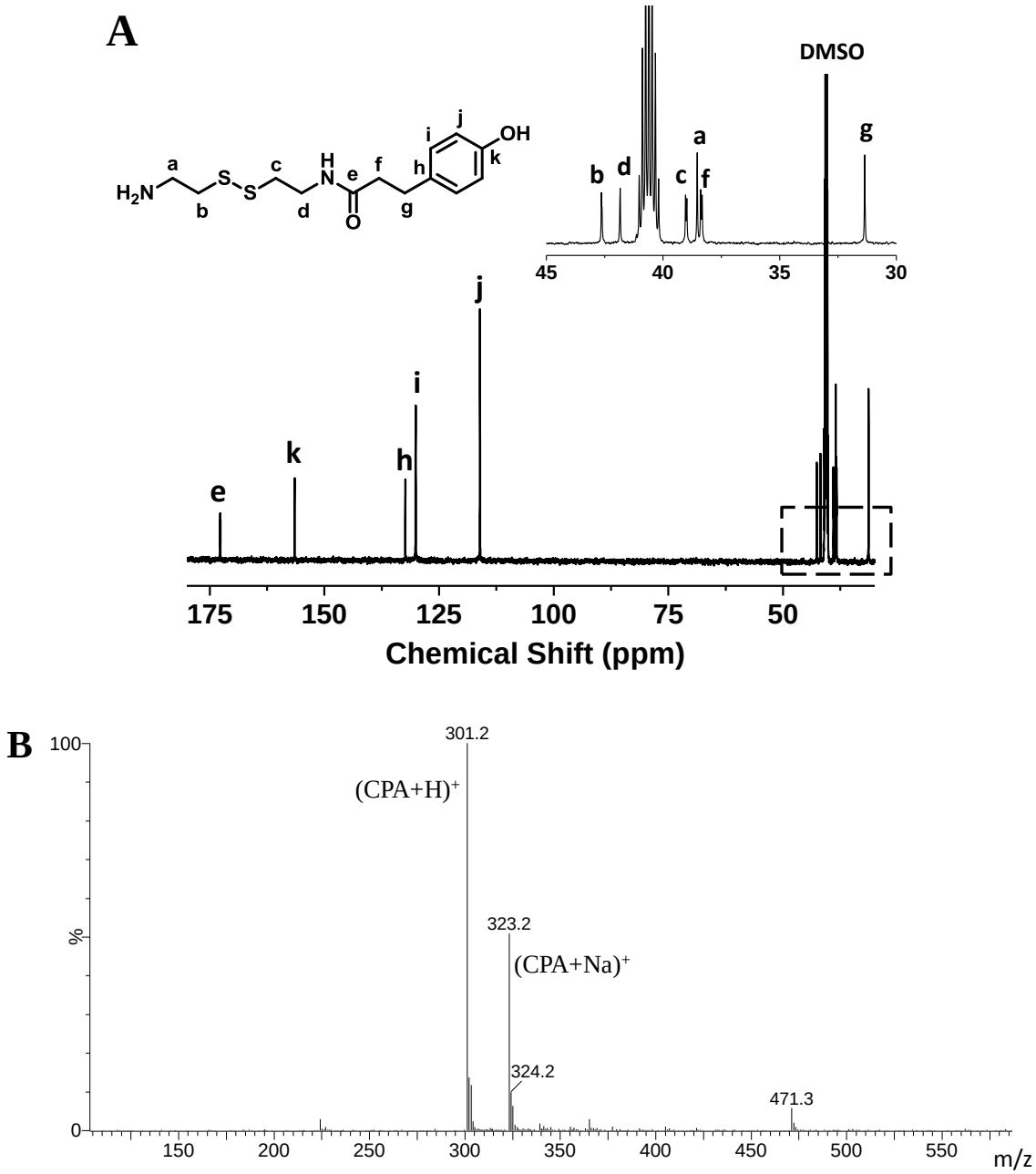


Figure S2. (A) ^{13}C NMR spectrum, (B) ES-MS of the synthesized CPA (M_W = 300.4).

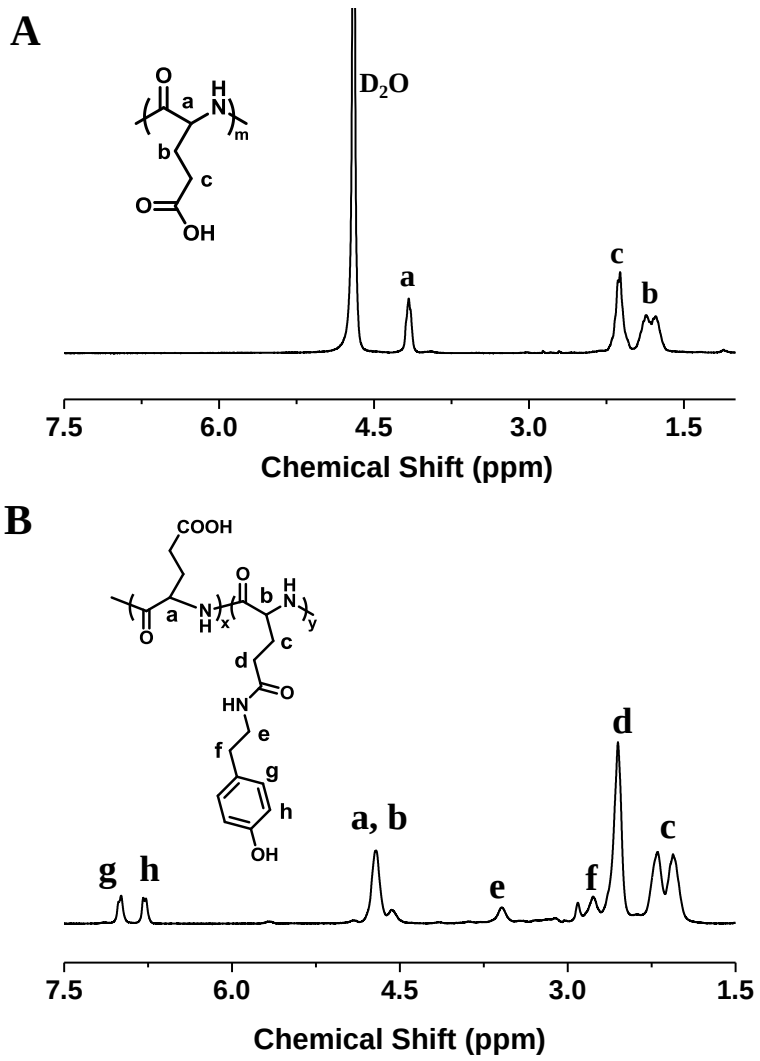


Figure S3. ^{1}H NMR spectra of (A) PLG in D_2O, (B) PLG-*g*-TA in CF_3COOD.

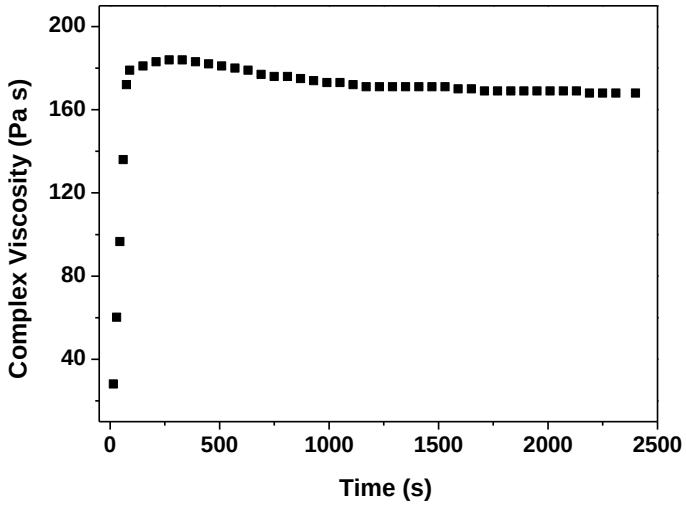


Figure S4. The change of complex viscosity with time of PLG-*g*-CPA solution (2% w/v) in presence of HRP (1 U/mL) and H_2O_2.

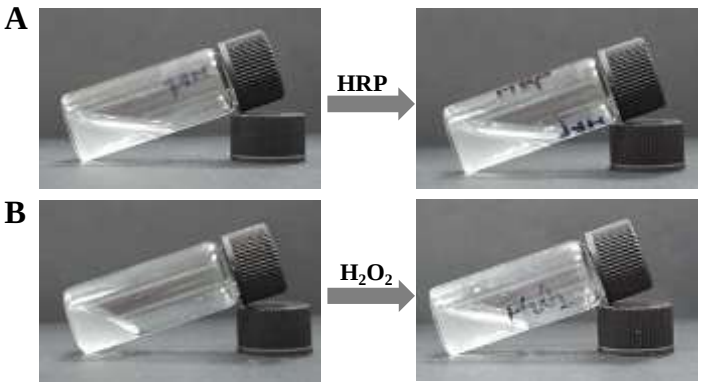


Figure S5. Photographs for the control experiments of hydrogel formation: (A) the photographs of the PLG-*g*-CPA solution with the addition of HRP only; (B) the photographs of the PLG-*g*-CPA solution with the addition of H_2O_2 only. The copolymer solutions could not transfer to hydrogel with HRP or H_2O_2 only.

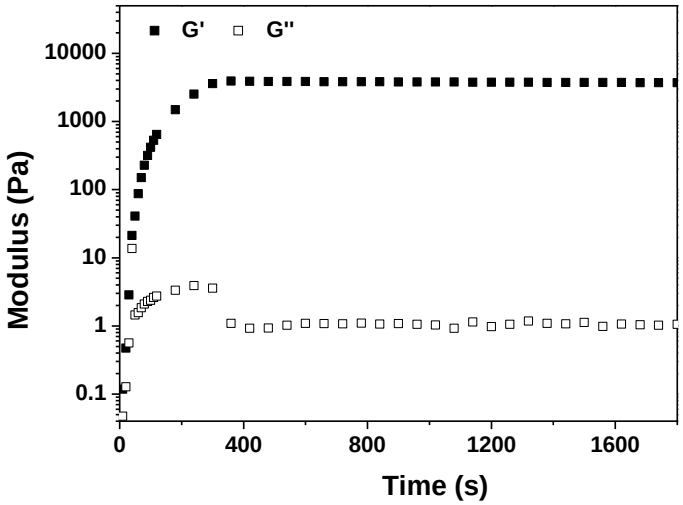


Figure S6. Storage modulus (G') and lose modulus (G'') of PLG-*g*-TA hydrogels (4% (w/v), HRP: 1 unit/mL, H_2O_2: 5mM).

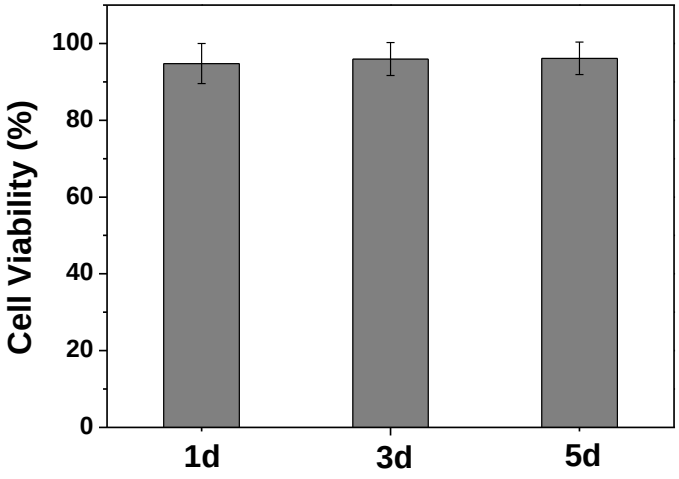


Figure S7. Viability of L929 cells after exposure to the eluants of the PLG-*g*-TA hydrogels (4% (w/v), HRP: 1 unit/mL, H_2O_2: 5mM) for 24 h, and the eluants were obtained after the hydrogel was cultured for 1, 3 and 5 days, respectively (n=3).

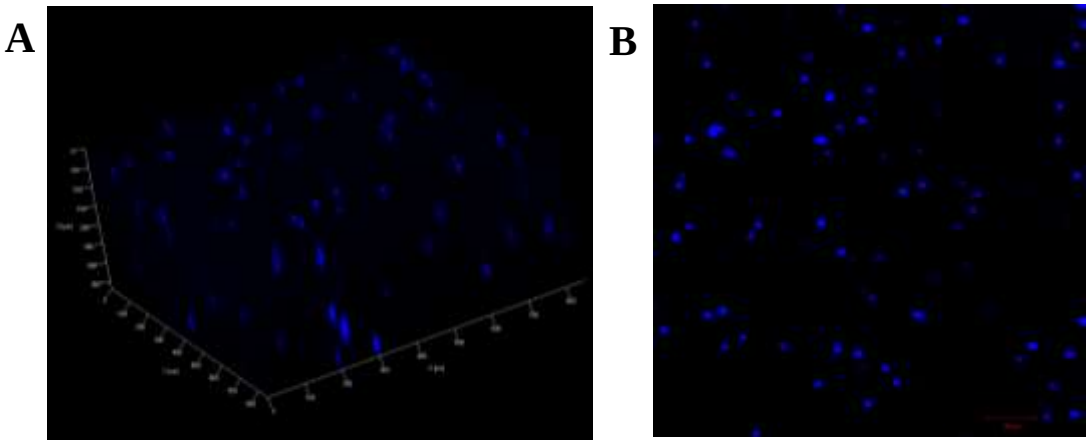


Figure S8. The images of L929 cells encapsulated in the PLG-*g*-TA hydrogels observed by the confocal laser scanning microscope (cells were stained with DAPI). (A) a stacked 3D image; (B) the longitudinal section image (scale bar: 100 μm).

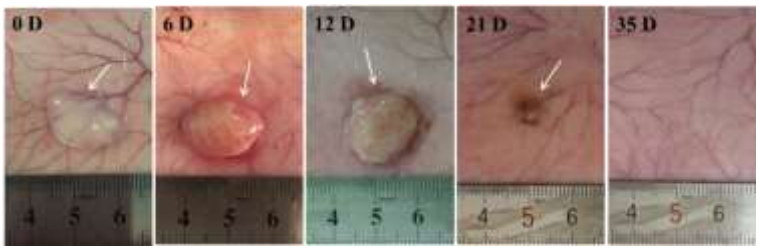


Figure S9. *In vivo* hydrogel status at different time intervals. PLG-*g*-TA hydrogels (0.5 mL, 4% (w/v)) were injected into the back of rats. Photos were taken at 15 min (0 day), and 3, 6, 12, 21 and 35 days after injection.

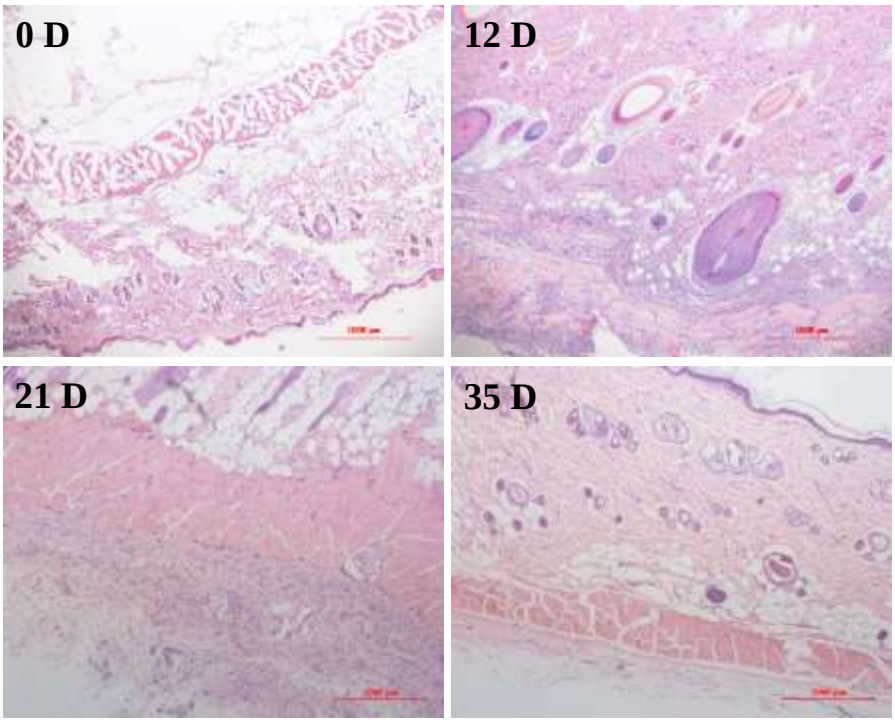


Figure S10. Tissue biocompatibility of the *in situ* formed PLG-*g*-TA hydrogels (H&E staining of tissues surrounding the injection sites at 15 min (0 day) and 12, 21, 35 days after injection).

References:

(1) He, C. L.; Zhao, C. W.; Guo, X. H.; Guo, Z. J.; Chen, X. S.; Zhuang, X. L.; Liu, S. Y.; Jing, X. B., Novel Temperature- and pH-responsive Graft Copolymers Composed of Poly(L-glutamic acid) and Poly(N-isopropylacrylamide). J. Polym. Sci., Part A: Polym. Chem. 2008, 46, 4140-4150.